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Separation Science: An Introduction to Chromatography

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ABSTRACT

This paper gives an overview on separation science: an introduction to chromatography. Chromatography is a lab technique used to separate mixtures into individual components. The term "chromatography" comes from the Greek word "chroma," meaning color. It's based on the principle that different components of a mixture interact differently with a stationary phase and a mobile phase, allowing them to be separated. The technique has a rich history, dating back to 1901 when Mikhail Semyonovich, a Russian botanist, first used it to separate plant pigments. Since then, chromatography has evolved, and various types have been developed, including: Paper chromatography, where a mixture is separated on a paper strip; Thin layer chromatography, which uses a thin layer of adsorbent; Column chromatography, where a mixture is separated in a vertical glass column Gas chromatography, which uses a gas as the mobile phase; Ion exchange chromatography, which separates compounds based on charge; Affinity chromatography, which uses selective interactions between molecules' Chromatography works on the principle of partitioning, where components of a mixture distribute themselves between the stationary and mobile phases. The retention time or retention factor is used to identify and quantify the separated components. Chromatography is widely used in various fields, including pharmaceuticals, biotechnology, and environmental science, for separation, purification, and analysis of complex mixtures.

Keywords Separation, protein, chromatography, theory, concept

1. Introduction

Chromatography is a laboratory technique used to separate, identify, and purify the components of a mixture. The term "chromatography" is derived from the Greek word "chroma," meaning color, and "graphy," meaning writing. This technique involves passing a mixture through a stationary phase, which separates the components based on their interactions with the stationary phase and a mobile phase. The concept of chromatography dates back to 1901 when Mikhail Semyonovich, a Russian botanist, first used it to separate plant pigments. Since then, chromatography has evolved, and various techniques have been developed, including paper chromatography, thin layer

chromatography, column chromatography, gas chromatography, and more (Santra, 2017; Karpek et al., 2024).

Chromatography relies on the principle of partitioning, where components of a mixture distribute themselves between the stationary and mobile phases. The stationary phase can be a solid or a liquid, while the mobile phase is typically a gas or a liquid. The interactions between the components and the phases can be based on differences in charge, adsorption, relative solubility, or size. Chromatography has become an essential tool in various fields, including pharmaceuticals, biotechnology, environmental science, and food analysis, due to its ability to separate and analyze complex mixtures. The technique has numerous applications, from purifying proteins to detecting environmental pollutants (Mujawar et al., 2024). Overall, chromatography is a powerful technique that has revolutionized the way we analyze and separate mixtures, and its importance continues to grow in various scientific disciplines. Chromatography has numerous applications across various fields, including:

- Pharmaceutical industry: purification and analysis of drugs, identification of impurities
- Biotechnology: separation and analysis of biomolecules like proteins, peptides, and nucleic acids
- Environmental science: detection and analysis of pollutants in air, water, and soil
- Food industry: analysis of food additives, contaminants, and nutrients
- Clinical chemistry: analysis of biological fluids like blood and urine for diagnosis
- Forensic science: analysis of evidence and identification of substances

Nevertheless, this paper gives an overview on separation science: an introduction to chromatography.

2. Chromatography

Chromatography is derived from the word "chroma" which entails color. Therefore, chromatography is a term elucidating laboratory techniques for separation of various mixtures. Chromatography is a laboratory technique that consists of a procedure involving passing a mixture (to be separated) containing an analyte through a given stationary phase, that is responsible for separating the analyte from other components of the mixture (Mujawar et al., 2024).

3. History

Chromatography was historically invented by Mikhail Semyonovich (a Russian botanist) in 1901 on the cause of conducting a study on chlorophyll. The scientist utilized a liquid adsorption column consisting of calcium carbonate to separate various plant pigments (Gulhane et al., 2022). Albeit, the chromatography was initially published in Congress of Naturalists and Doctors in St. Petersburg, the first printed version of the technology was seen in 1903 in the Proceedings of the Warsaw Society of Naturalists (biology section). The term chromatography was firstly found in

1906 in the papers published by Beriche de Deutschen Botanischen Gesellschaft (a German botanical journal). In 1907, the term chromatograph was demonstrated to the German Botanical Society. By the 1952, Mr Archer Porter Martin and Mr Richard Laurence Millington Synge prosperously received Chemistry Nobel Prize for inventing partition chromatography and afterwards a lot of historical advancements in chromatography technology were recorded (Karpek et al., 2024).

4. Chromatographic techniques and their terms

There are some definitive terms that are utilized in describing chromatography; some of the major ones are supplied as follows:

Analyte - An analyte is the substance intended to be isolated or purified during chemotherapy

Chromatograph- Chromatograph is responsible for taking a chemical mixture carried by gas or liquid and ensures its separation into various components over a stationary phase. The separation takes account the varied differences in features of the mixture components.

Chromatogram- A chromatograph is the visual outcome (output) of the chromatograph. Different components in a mixture correspond to varied different peaks appearing in a chromatograph.

Retention time- Retention time refers to the time it takes a given molecule to pass through the Chromatography system.

Stationary phase- Stationary phase entails the fixed component in chromatography procedure. It is the phase whereby the solvents and analyte (mixture) travels through. For instance, silica plate is a typical stationary phase utilized in thin layer chromatography (Mujawar et al., 2024).

5. Theory

The chromatography theory relied on the fact that, chromatography is a separation method or technique that capitalized on the differences in partitioning behaviors between mobile and stationary phase to purify components of a mixture. The idea of interaction between stationary phase and mixture (analyte) could be due differences in charge, adsorption, relative solubility, etc (Mujawar et al., 2024). Two chromatography theories are identified here:

Plate theory - The plate theory was initiated by Archer John Porter Martia and works of Richard Laurence Millington Synge, therefore, the theory postulates that the chemotherapy system has the mobile and stationary phases in equilibrium, thus the partition K is given by = Concentration of solute in stationary phase/concentration of solute in mobile phase.

Retention theory - The retention theory, the retention is a measure of the speed at which the substance travels in a chromatographic system. In new methods such as Gas Chromatography, whereby the compounds released along with the eluent, the retention is appraised as retention time

R_t, as the time between injection and detection. In other opposite methods of chromatography, the retention is appraised in terms of retention factor (R_f) which is given by=

$R_f = \text{Distance moved by compound} / \text{Distance moved by solvent}$ (Gupta et al., 2020).

6. Types of chromatography

Countercurrent chromatography- Is a type of chromatography technique consisting of liquid-liquid approach, therewith, both the mobile and stationary phase are in liquid form. In this methodology, solutions are mixed and later allowed to settle into layers that are subsequently separated.

Paper chromatography -This is an old technology whereby a small portion of liquid mixture is spotted on a strip chromatography paper. Thus, the paper has to be dipped into a convenient solvent and allowed in a sealed container, therefore the solvent rises through the paper to meet the mixture. This meeting allowed the mixture to travel up the paper along with the solvent. Invariably, the samples in the mixture travel at different rate along the paper strip(Gupta et al., 2020).

Thin layer chromatography - This method is relevant to paper chromatography, whereby the paper strip is substituted by thin layer adsorbent such as silica gel, cellulose, and alumina. This is an updated method with more speed, more separation, and optional value in selecting the stationary phase materials. Invariably, different compounds present in the mixture had to travel according to their specific difference.

Column chromatography - This method involves the use of a typical vertical glass column containing a solid support, with the sample on the support, for the sample to travel through the column under the influence of gravity. Different samples components travel differently at different exit time. Flash column chromatography is an updated version that involves application of pressure that encourages separation in less than 20 minutes (Gupta et al., 2020).

Fast protein chromatography is a liquid chromatography and is applicable in separation of protein substances.

Gas Chromatography- Gas Chromatography is according to the fact that a partition equilibrium of analyte is established between a liquid stationary phase and a mobile gas. The stationary phase cling to the inner part of a small-diameter glass tube (otherwise capillary column) or solid matrix in a larger metal tube (Karpek et al., 2024).

Ion exchange chromatography- Ion exchange chromatography is a type of column chromatography that utilized a charged material as a stationary phase. This method is applicable in separation of compounds like amino acids, proteins, and peptides.

Affinity chromatography - This is a method that enjoyed the selective noncovalent interaction between an analyte and given molecules.

Size exclusion chromatography - Size exclusion chromatography is type of chromatography that applied differences in size of molecules to separate them. Smaller molecules travel through a porous medium. It is utilized as polishing step in the separation of tertiary structure, and quaternary structure of proteins after purification (Gulhane et al., 2022).

7. Conclusion

Chromatography is a powerful laboratory technique used for separating and analyzing complex mixtures. Its versatility and range of applications have made it an essential tool in various fields, including pharmaceuticals, biotechnology, environmental science, and more. From its inception in 1901 by Mikhail Semyonovich to the present day, chromatography has evolved significantly, with various types and techniques being developed to suit specific needs. The technique's ability to separate components based on differences in partitioning behaviors, charge, adsorption, and size has made it invaluable for purification, identification, and quantification of substances. As research and technology continue to advance, chromatography is likely to remain a crucial component of scientific inquiry and discovery.

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